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ance Notes on Codes and Abbreviations" appearing at the begin-
ning of each regular issue of the PCT Gazette.*

(54) Title: ISOLATION AND USE OF RYANODINE RECEPTORS

(57) Abstract: The genes encoding ryanodine receptor homologs have been characterized from multiple insect families including lepidopteran tobacco budworm (*Heliothis virescens*), homopteran green peach aphid (*Myzus persicae*), corn plant hopper (*Peregrinus maidis*), cotton melon aphid (*Aphis gossypii*) and fruitfly (*Drosophila melanogaster*). The full-length genes have been isolated, cloned and amplified in bacterial cells. Expression in insect cells shows that the recombinant protein folds into a functional calcium release channel. The genes and their corresponding polypeptides have a number of uses including, but not limited to, the isolation of other pest ryanodine receptors, the development of screens to identify insecticidally active compounds, use of fragments of genes as pesticides, fragments of protein for antibody production, fragments of protein for determination of the structure of insecticide binding sites, and identification of insecticides that disrupt the calcium balance in cells through other messengers that interact with the receptor calcium release mechanism. Methods are outlined for overcoming toxic effects of expressing recombinant proteins in host cells.



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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US03/29834

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : C07K 1/00, 14/00, 17/00; C07H 21/02, 21/04; C12Q 1/00; G01N 33/53; C12N 15/00, 15/09, 15/63, 15/70
US CL : 536/23.1; 530/350; 435/4, 7.1, 320.1

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 536/23.1; 530/350; 435/7.1, 320.1, 455

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Continuation Sheet

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 01/71042 A2 (PE CORPORATION) 27 September 2001 (27.09.2001), see whole document, especially pages 1-7.	1-15
A	TAKESHIMA et al. Isolation and characterization of a gene for a ryanodine receptor/calcium release channel in drosophila melangoaster. FEBS Letters. 1994, Vol. 337, pages 81-87.	1-9, 11-15
A	US 2001/0046664 A1 (MURPHY et al.) 29 November 2001 (29.11.01), see whole document, especially pages 11 and 13-14.	1-9, 11-16, 19, 20



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T"

later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X"

document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y"

document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

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document member of the same patent family

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Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claim Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claim Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claim Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:
Please See Continuation Sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.: 1-16, 18, 19, 20 (SEQ ID NOs: 1 and 2)

Remark on Protest

☐
☐

The additional search fees were accompanied by the applicant's protest.

No protest accompanied the payment of additional search fees.

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING

This application contains the following inventions or groups of inventions, which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

Group I, claim(s) 1-16, 18, 19, 20, drawn to an isolated nucleotide sequence encoding an amino acid sequence identity of at least 80% when compared to a polypeptide consisting of SEQ ID NO: 2, an isolated nucleic acid sequence of SEQ ID NO: 1, construct and transformed host cell comprising said nucleotide sequence and a method of evaluating at least compound for its ability to modulate homeostasis using said host cell.

Group II, claim(s) 1-16, 18, 19, 20, drawn to an isolated nucleotide sequence encoding an amino acid sequence identity of at least 80% when compared to a polypeptide consisting of SEQ ID NO: 4, an isolated nucleic acid sequence of SEQ ID NO: 3, construct and transformed host cell comprising said nucleotide sequence and a method of evaluating at least compound for its ability to modulate homeostasis using said host cell.

Group III, claim(s) 1-16, 18, 19, 20, drawn to an isolated nucleotide sequence encoding an amino acid sequence identity of at least 80% when compared to a polypeptide consisting of SEQ ID NO: 8, an isolated nucleic acid sequence of SEQ ID NO: 7, construct and transformed host cell comprising said nucleotide sequence and a method of evaluating at least compound for its ability to modulate homeostasis using said host cell.

Group IV, claim(s) 1-16, 18, 19, 20, drawn to an isolated nucleotide sequence encoding an amino acid sequence identity of at least 80% when compared to a polypeptide consisting of SEQ ID NO: 10, an isolated nucleic acid sequence of SEQ ID NO: 9, construct and transformed host cell comprising said nucleotide sequence and a method of evaluating at least compound for its ability to modulate homeostasis using said host cell.

Group V, claim(s) 17 and 18, drawn to a method for evaluating at least one compound which modulates a ryanodine receptor activity, the method comprising contacting at least one compound with a polypeptide encoded by the nucleic acid sequence set forth in SEQ ID NO: 2.

Group VI, claim(s) 17 and 18, drawn to a method for evaluating at least one compound which modulates a ryanodine receptor activity, the method comprising contacting at least one compound with a polypeptide encoded by the nucleic acid sequence set forth in SEQ ID NO: 4.

Group VII, claim(s) 17 and 18, drawn to a method for evaluating at least one compound which modulates a ryanodine receptor activity, the method comprising contacting at least one compound with a polypeptide encoded by the nucleic acid sequence set forth in SEQ ID NO: 8.

Group VIII, claim(s) 17 and 18, drawn to a method for evaluating at least one compound which modulates a ryanodine receptor activity, the method comprising contacting at least one compound with a polypeptide encoded by the nucleic acid sequence set forth in SEQ ID NO: 10.

Group IX, claim 21, drawn to an isolated nucleic acid fragment encoding a combination of polypeptides set forth in SEQ ID NO: 63-119. The first combination is SEQ ID NO: 63 and 64. The number of possible combinations is 561 "functional". If applicants want additional combination(s) to be searched, applicants are required to define the combination and pay an additional search fee for each single combination of 2 or more polypeptides because each combination is considered drawn to a single invention. Each additional combination is an additional search fee of \$210.

Group X, claims 22 and 23, drawn to a method for identifying a nucleic acid sequence encoding an insect ion channel comprising obtaining an isolated nucleic acid encoding a first polypeptide having at least 100 amino acids and comparing a polypeptide selected from the group consisting of SEQ ID NOs: 63-119. The first combination is SEQ ID NO: 63 and 64. The number of possible combinations is 561 "functional".

If applicants want additional combination(s) to be searched, applicants are required to define the combination and pay an additional search fee for each single combination of 2 or more polypeptides because each combination is considered drawn to a single invention. Each additional combination is an additional search fee of \$210.

Group XI, claim(s) 24, 27, 28, 29, 32, and 33, drawn to a method for expressing an isolated nucleic acid fragment encoding a toxic insect ion channel which comprises transforming a host with a recombinant construct comprising in the 5' to 3' direction a promoter operably linked to the toxic insect ion channel nucleic acid wherein the promoter comprises a transcription termination nucleic acid fragment situated between said promoter and the isolated nucleic acid fragment encoding the toxic insect ion channel.

Group XII, claim(s) 25, 27, 28, 30, 32, and 33, drawn to a method for expressing an isolated nucleic acid fragment encoding a toxic insect ion channel which comprises transforming a host with a recombinant construct comprising in the 5' to 3' direction a promoter operably linked to the toxic insect ion channel nucleic acid wherein the promoter comprises a nucleic acid fragment comprising at least one in-frame translational termination codon situated between said promoter and the isolated nucleic acid fragment encoding the toxic insect ion channel.

Group XIII, claim(s) 26, 27, 28, 31, 32, and 33 drawn to a method for expressing an isolated nucleic acid fragment encoding a toxic insect ion channel which comprises transforming a host with a recombinant construct comprising in the 5' to 3' direction a promoter operably linked to the toxic insect ion channel nucleic acid wherein the promoter comprises a nucleic acid fragment consisting essentially of at least one transcription termination nucleic acid fragment and at least one in-frame translational termination codon situated between said promoter and the isolated nucleic acid fragment encoding the toxic insect ion channel.

This application contains claims directed to more than one species of the generic invention. These species are deemed to lack unity of invention because they are not so linked as to form a single general inventive concept under PCT Rule 13.1.

In order for more than one species to be examined, the appropriate additional examination fees must be paid. The species are as follows:

- a) SEQ ID NO: 128, 130, 144, and 146 in claim 1.
- b) SEQ ID NO: 127, 129, 143, and 145 in claim 6.

The claims are deemed to correspond to the species listed above in the following manner:

Claims 1, 2, 3, 4, 5, 7, 8, 9, 12, 13, 14, 15, 16, 19, and 20 correspond to the species of a).

The following claim(s) are generic: claims 1 and 11.

Claim 6 corresponds to the species of b).

The following claim(s) are generic: Claim 6.

The inventions listed as Groups I-XIII do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

The special technical feature of Group I is considered to be drawn to SEQ ID NO: 2.

The special technical feature of Group II is considered to be drawn to SEQ ID NO: 4.

The special technical feature of Group III is considered to be drawn to SEQ ID NO: 8.

The special technical feature of Group IV is considered to be drawn to SEQ ID NO: 10.

The special technical feature of Group V is considered to be drawn to a method for evaluating at least one compound which modulates a polypeptide consisting of at least 80% when compared to SEQ ID NO: 2.

The special technical feature of Group VI is considered to be drawn to a method for evaluating at least one compound which modulates a polypeptide consisting of at least 80% when compared to SEQ ID NO: 4.

The special technical feature of Group VII is considered to be a method for evaluating at least one compound which modulates a polypeptide consisting of at least 80% when compared to SEQ ID NO: 8.

The special technical feature of Group VIII is considered to be a method for evaluating at least one compound which modulates a polypeptide consisting of at least 80% when compared to SEQ ID NO: 10.

The special technical feature of Group IX is considered to be an isolated nucleic acid fragment encoding an insect ion channel comprising at least two polypeptide sequences set forth in any of SEQ ID NOs: 63-119. The common structural feature for SEQ ID NOs: 63-119 is

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not disclosed in the disclosure. Therefore, the technical feature linking the combination of two or more polypeptides lack the same or corresponding technical feature.

The special technical feature of Group X is considered to be a method of identifying a nucleic acid sequence encoding an insect ion channel comprising comparing a polypeptide to a polypeptide selected from the group consisting of SEQ ID NOs: 63-119. The common structural feature for SEQ ID NOs: 63-119 is not disclosed in the disclosure. Therefore, the technical feature linking the combination of two or more polypeptides lack the same or corresponding technical feature.

The special technical feature of Group XI is considered to be a method of using a construct comprising a promoter comprising a transcription termination nucleic acid fragment.

The special technical feature of Group XII is considered to be a method of using a construct comprising a promoter comprising at least one in-frame translational termination codon.

The special technical feature of Group XIII is considered to be a method of using a construct comprising a promoter comprising a transcription termination nucleic acid fragment and at least one in-frame translational termination codon.

Accordingly, Groups I-XIII are not so linked by the same or a corresponding special technical feature as to form a single inventive concept

The species listed above do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, the species lack the same or corresponding special technical features for the following reasons: the species of (a) the amino acid sequences are different structurally and the disclosure does not disclose a common structural feature for the amino acid sequences; (b) the nucleotide sequences are different structurally and the disclosure does not disclose a common structural feature for the nucleotide sequences.

Continuation of B. FIELDS SEARCHED Item 3:

WEST2.1, STN

search terms: ryanodine receptor, construct, calcium homeostasis, cell, oligomer, ion channel